Ligation Protocol

1. Add 2 µL of each restriction digest product to the reaction tube.
2. Add 2 µL of T4 DNA Ligase buffer to the tube.
3. Add 1 µL of ligase to the tube.
4. Add 11 µL of water to the tube.
5. Allow the reaction to proceed at room temperature for one hour.

Component	Volume
Part A Digest	2 uL
Part B Digest	2 uL
Linearized Plasmid Digest	2 uL
T4 DNA Ligase Buffer	2 uL
DNA Ligase	1 uL
dH2O	11 uL
Total	20 uL

*****If this ligation protocol does not work, equimolar amounts of part A and part B were not added. Therefore, part A and part B volumes can be changed to obtain the correct ligation product. Total volume should be kept at 20 uL.**